Community-Acquired Pneumonia: Bacteriological Profile and Microbiological Investigations

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Introduction

Lower respiratory tract infections (LRTIs) are a leading cause of death due to infectious diseases worldwide. Of the LRTIs, community-acquired pneumonia (CAP) is the most important infectious disease of the respiratory tract encountered in clinical practice. It has an estimated incidence ranging from 1.6/1,000 to 16/1,000 per year.¹,²

Diagnostic testing at one point of time was critical to antimicrobial agent selection, clinical outcomes, and performance measures for management of the patient. However, over the past couple of decades, there has been an extraordinary decline in interest and supposed need for microbiological analysis in CAP cases. Also, the advent of antimicrobial agents to treat CAP has led to a progressive decrease in the perceived requirement to know the causative agent for which the patient is being treated.¹

The microbiology of CAP may have probably remained stable over the past decade. However, there remains a possibility of change over time owing to an aging population and the success of vaccinations.² The vast number of CAP studies from the pre-penicillin era showed that an etiologic diagnosis was established in > 90% of cases. Contrary to this, the 2009 data from Medicare indicate that a probable pathogen is now detected in < 10% based on a review of the records of > 17,000 patients hospitalized with CAP. The desire for better data-directed treatment toward pathogens clashes with harsh realities of cost, requirements for antibiotics to be given within 6 hours of disease onset, guidelines discouraging microbiological studies in most cases, and beliefs in empirical treatment approaches among others.¹

While the initial antibiotic selection for CAP treatment remains empirical, consensus exists on the fact that selection of appropriate antimicrobial agents in CAP management is notably simplified if the pathogen is defined.¹,² Additionally, given that the most of the forms of CAP are treatable, a better understanding of the causative pathogens could help outline efforts to define their natural history and optimize treatment.²

Common Etiologies of Community-acquired Pneumonia

Microbial etiology of CAP is not yet well-characterized.³ Each case of CAP shows a variation in the causative agent/s implicated in its pathogenesis, which can be attributed to the regional differences in the prevalence of microorganisms. Nevertheless, there continue to be several common etiologies that lead to CAP both in the hospital and community settings.³

The role of viruses in adult CAP is not well-understood. They have been previously encountered in approximately 10% of adult patients admitted to hospital with CAP. However, studies based on molecular diagnostics indicate an underestimation of the viral etiology in adult CAP owing to a previously limited range of diagnostic methods. The incidence of mixed infections involving viruses and respiratory pathogens is suggested to be common among patients hospitalized for CAP.³

The common pathogens implicated to cause pneumonia in ambulatory patients are Streptococcus pneumoniae (S. pneumoniae), Mycoplasma pneumoniae (M. pneumoniae), Haemophilus influenzae (H. influenzae), Chlamydia pneumoniae (C. pneumoniae), and respiratory viruses, such as influenza A and B, adenovirus, respiratory syncytial virus, and parainfluenza. The common pathogens causing pneumonia in hospital settings include S. pneumoniae, M. pneumoniae, H. influenzae, C. pneumoniae, Legionella spp., and aspiration respiratory viruses, such as influenza A and B, adenovirus, respiratory syncytial virus, and parainfluenza. Severe pneumonia is caused by the pathogens S. pneumoniae, Staphylococcus aureus (S. aureus), Legionella spp., Gram-negative bacilli, and H. influenzae.⁴ Atypical bacteria are predominant in patients with milder episodes of pneumonia and cases of ambulatory CAP.²

Streptococcus pneumoniae causes an estimated 1.6 million deaths worldwide. It is the most common pathogen causing CAP and an important reason for morbidity and mortality in the elderly, followed by S. aureus and Pseudomonas aeruginosa (P. aeruginosa).⁵,⁶ Streptococcus pneumoniae also happens to be the most common etiologic agent isolated in CAP patients with severe disease and those requiring hospitalization. According to the population-based studies, S. pneumoniae was also the organism frequently isolated in outpatients with evidence of a similar etiologic profile across all age groups (both inpatients and outpatients).³ A detailed understanding of the pneumococcal epidemiology is essential, as the available vaccines target the polysaccharide capsule or the serotype-specific capsular protein.⁵

New and Emerging Etiologies of Community-acquired Pneumonia

In addition to the above mentioned common pathogens causing CAP, few studies undertaken have highlighted on the new and emerging etiologies of CAP that can influence the management of the condition.⁴

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A prospective, multicenter study carried out by Fang et al. in 359 cases found *S. pneumoniae* (15.3%), *H. influenzae* (10.9%), *Legionella* spp. (6.7%), and *C. pneumoniae* (6.1%) to be the common causative pathogens of CAP. The highest mortality was seen with *S. aureus* (50%) and the lowest with *C. pneumoniae* (4.5%). The study highlighted on *C. pneumoniae* and *Legionella* spp. as possible emerging etiologies for CAP and the need for the observation to influence empiric antibiotic prescriptions.

A new diagnostic platform using polymerase chain reaction (PCR) along with conventional methods evaluated the microbiological yield with an aim to identify emerging etiologies for CAP in a 12-month prospective study. Conventional testing included the culture of blood, sputum, and nasopharyngeal secretions. Other tests included analyses of sputum samples using real-time, quantitative PCR for *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis* [*M. catarrhalis*]; nasopharyngeal secretion analysis using PCR; serological testing for *M. pneumoniae*, *C. pneumoniae*, and respiratory viruses; and the detection of pneumococcal and *Legionella pneumophila* [*L. pneumophila*]) antigens using urine antigen assays.

Adoption of the above mentioned approach helped to establish microbial etiology in about 67% of patients and identify a microbiological agent in 89% of them. The most frequently detected pathogens were *S. pneumoniae* (38%) and respiratory virus (29%). About 35% of patients were identified to be infected with two or more pathogens.

Data from a recent case series have highlighted on methicillin-resistant *S. aureus* to be a cause for severe CAP. However, it remains an uncommon cause, and the prevalence and risk factors are not known.

### Bacteriological Profile of Community-acquired Pneumonia

The bacteriological profile of CAP is seen to vary across countries and within the regions of the same country. This fact is attributed to factors like environmental pollution, differences in frequency of use of antibiotics, awareness of the disease, and life expectancy. However, the most common pathogen across countries, such as India, Europe, the United Kingdom, and the United States, is *S. pneumoniae*.

#### Indian scenario

Etiologic agents of CAP vary across the different geographical areas of the country. *Streptococcus pneumoniae* predominates as the etiologic agent in Shimla and Delhi. Other frequently isolated pathogens identified in about 75% of patients from Shimla were *S. aureus*, *Klebsiella pneumoniae*, *K. pneumoniae*, *M. pneumoniae*, *Escherichia coli* (*E. coli*), and other Gram-negative bacteria. The organisms isolated from CAP patients in a prospective study conducted in Mumbai included *S. pneumoniae*, *C. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *M. pneumoniae*, *L. pneumophila*, *P. aeruginosa*, *Staphylococcus*, and *Salmonella typhi*. *Streptococcus pneumoniae* was the leading cause of CAP, isolated in 22% of patients; while atypical organisms and *Mycobacterium tuberculosis* were identified in 19% and 7% of patients, respectively.

In a study among hospitalized patients, *S. aureus* was the primary causative organism identified. Other pathogens identified included isolates of *Pseudomonas* and *Klebsiella*. In critically ill patients, most likely isolates include Enterobacteriaceae, *S. aureus*, and *P. aeruginosa*. In yet another study recently conducted in 2015 among CAP patients in Mumbai, the common organisms isolated from sputum culture were as given in Figure 1. *Streptococcus pneumoniae*, *Pseudomonas*, *E. coli* and *Acinetobacter*, *S. aureus*, *Klebsiella*, and mixed infections were found in 56%, 11.1%, 5%, 3.3%, 1.3%, and 16.7% of patients, respectively.

### Tests to Determine Etiology of Community-acquired Pneumonia

The knowledge of pathogens that cause CAP is the basis for the selection of empirical antibiotic treatment that in turn has a substantial impact on the patient prognosis. However, the establishment of a microbial diagnosis for patients with CAP continues to pose a challenge. The current guidelines on CAP discourage the approach based on microbiological diagnosis for pneumonia in non-hospitalized patients, which is probably due to lacking sensitive, specific, and cost-effective diagnostics. Although retrospective studies may have revealed favorable outcomes using empiric therapy in CAP patients, failing to accurately identify the causative agent as *S. pneumoniae* has far-reaching consequences both for the affected individual and the population as a whole. At the patient level, it may result in an inadequate antimicrobial therapy or overly broad-spectrum and expensive treatment, translating into limitations in assessing disease burden, evaluating the effects of immunization, tracking of antimicrobial resistance, and exploring new therapeutic agents among the population.

Traditionally, the diagnosis of CAP has been made using the conventional method of culture of respiratory
These diagnostic approaches. However, microbiological samples, such as sputum, are subject to oropharyngeal contamination, and hence, pose difficulty in detecting relatively fastidious bacteria like *S. pneumoniae*. While some laboratories continue to perform traditional methods of Gram staining and culture or Quellung staining to distinguish *S. pneumoniae*, these approaches have become relatively unusual. The sensitivity and specificity of sputum Gram stain vary among the different settings. Compared to culture, the sensitivity of this test ranges between 10% and 15%, while its specificity ranges from 11% to 100%. Gram staining and culture to identify *S. pneumoniae* is problematic as a result of false-positive and false-negative results.

As against the pneumococci, *S. aureus* and Gram-negative bacteria are easily detected using selective media. Although rare, these organisms are likely to be disproportionately represented, especially in specimens obtained post-treatment with antibiotics.

A sputum culture cannot differentiate pathogen from commensal and is of no value if obtained after starting antibiotics, as it will only lead to the detection of colonizers. Identification of microbe is possible only in 50% of patients and depends on the quality of sputum sample.

**Urinary antigen test**

Detecting pneumococcal antigen in urine is the most widely used indirect method for *S. Pneumoniae* detection of pneumococcal antigen in urine. The group C polysaccharide cell wall antigen is detected by this test. It is useful in diagnosing pneumococcal pneumonia. It is a quick, sensitive, and specific test to detect pneumococcal CAP in adults and it may remain positive for many weeks after pneumococcal pneumonia.

The advantages include ease of getting a diagnostic specimen, greater diagnostic yield compared to sputum, and ability to establish the diagnosis even after antibiotic treatment initiation. The test as evaluated in studies showed a sensitivity of 82% and a specificity of 97%. The sensitivity and specificity are, however, less in adults with non-bacteremia pneumonia. The main disadvantage noted with this test is its false-positive results in patients with nasopharyngeal colonization and those with recent episodes of pneumococcal infections. However, it is the favored method for the detection of Legionnaires disease.

The assay has a significant role to play in the rapid etiologic diagnosis of CAP, particularly in patients unable to produce good quality sputum, who are not bacteremic, and in whom antibiotics have been administered before admission. It significantly increases the diagnosis of a pneumococcal origin beyond standard microbiological cultures. It raises the intriguing possibility that considerably more cases of CAP are caused by *S. pneumoniae* than that conventional tests can currently confirm (Figure 2).

**Molecular diagnostics**

The traditional microbiological methods for the detection of respiratory tract pathogens can be slow. They are often not sensitive, fail to differentiate infection from colonization, and are influenced by previous antibiotic therapy. Molecular diagnostics are more promising in the detection of the common and atypical bacterial pathogens that can cause CAP. Molecular analysis can detect typical pathogens in hours and atypical pathogens in weeks. This approach aids to eliminate concerns about decreased viability of the organism associated with the effects of previous antibiotic therapy and transport of specimens.

Current molecular diagnostic methods have a great potential to include targets useful in the rapid identification of microorganisms and antimicrobial resistance. They aid to analyze directly unprocessed samples.
samples and to obtain quantitative results in pneumonia. Molecular methods compared to traditional methods have superior sensitivity, relatively rapid turnaround time, and are able to identify pathogens that are slow growing or difficult to culture. A variety of respiratory samples including expectorated sputum, bronchoalveolar lavages, protected bronchial brushes, and endotracheal aspirates are amenable to molecular testing. These assays may target either a single pathogen or multiple respiratory pathogens in a single assay.\(^4\)

Nucleic acid detection with the use of PCR has the advantage of being able to identify pathogens in patients already on treatment with antibiotics. Molecular and antigen-based testing possess the advantage of being able to deliver rapid results before initiation of treatment.\(^5\) Furthermore, PCR is useful to identify atypical bacterial strains, including Mycoplasma, C. pneumoniae, and H. influenzae type b. It is more rapid as compared to virus isolation and serology and is also more sensitive. Of recent, the FDA has cleared the PCR assay for the detection of 12 respiratory tract viruses. Rapid diagnostic tests for detecting influenza viruses show good specificity but a sensitivity of only 50–70%.\(^15\)

Conventional PCR, real-time PCR, and in-house or commercial multiplex mPCR are considered to be more specific than cultures and serology. A mPCR method compared the diagnostic yields that were obtained from mPCR of nasopharyngeal aspirates, nasopharyngeal swabs, and induced sputum (IS) with those obtained with specific PCR commercial kits, paired serology, and urinary antigen. All the PCR's were seen to have good specificity but low sensitivity in nasopharyngeal samples. The IS sample had the best performance for the diagnosis of M. pneumoniae by PCR. Sensitivity and specificity of upto 100% was stated to be obtainable if the characteristics of the population, the type of respiratory sample, and the inhibitors present in each of them is known. These tests, however, did not allow for discrimination between patients with acute or convalescent infection and asymptomatic carriers.\(^16\)

Implementation of comprehensive molecular testing of single lower respiratory tract specimens achieved pathogen detection in 87% of patients (adults hospitalized with CAP) as compared to 39% with culture-based methods. There was significantly greater detection of H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus, E. coli, and K. pneumoniae than standard culture-based methods. Polymerase chain reaction significantly detected bacteria in 143 culture-negative specimens. In addition a higher bacterial load was detected in culture-positive specimens as compared to culture-negative specimens. It also provided valuable information about individual bacterial loads. It was seen to significantly improve pathogen detection in CAP, especially in patients exposed to antimicrobials and enabled early de-escalation from broad-spectrum empirical antimicrobials to pathogen-directed therapy. Reporting of results was done in a clinically relevant time-frame.\(^17\)

In yet another study two diagnostic bundles that included sputum and blood culture, urinary antigen detection, and nasal swabs for PCR probes and at least one measurement of procalcitonin level were compared in patients (127 in number) with CAP. While an etiologic diagnosis was established in 71% of patients, a respiratory virus was detected in 39%. The potential of molecular diagnostics and serum procalcitonin (PCT) levels for improved antibiotic stewardship was evident in 25 patients with only detectable respiratory virus and normal levels of PCT.\(^18\)

Some of the contributions of these novel molecular diagnostic approaches include multiplex assays, user-friendly formats, providing of results in a few hours, high sensitivity and specificity in identification of pathogens, detection of antibiotic resistance genes, and target quantification. Future challenges may include the development of new molecular tests for other bacterial respiratory pathogens, detection of pathogens and new key antimicrobial resistance genes in unprocessed samples, and determination of the microbial load by quantitative multi-pathogen tests.\(^19\)

**Serologic tests for community-acquired pneumonia**

The immunoglobulin M (IgM) antibodies against Mycoplasma are seen to rise one week after infection and persist to be positive for 6 months to 1 year after infection, resulting in poor sensitivity and specificity of the test. It takes 3 weeks for IgM antibodies to Chlamydia to become detectable, resulting in poor sensitivity. Mycoplasma can be diagnosed by cold agglutinins (sensitivity of 50% and poor specificity) and elevated reticulocyte count. The limitations are that it requires rising titers, is expensive, and is not readily available.\(^4\)

**Antibody tests**

Diagnostic tests may include the detection of antibodies to S. pneumoniae, Mycoplasma, Chlamydia, adenovirus, influenza viruses (A and B), parainfluenza viruses (1, 2 and 3), respiratory syncytial virus, etc.\(^15\)

**Spectrum of Pneumococcal Disease**

Pneumococcal disease may be classified as invasive or non-invasive disease. Pneumonia, otitis media, and sinusitis are the classical presentations of non-invasive diseases. Invasive disease is characterized by sepsis, bacteremic pneumonia, meningitis, and arthritis.\(^4\) Streptococcus pneumoniae, a Gram-positive, catalase-negative organism, is responsible for pneumococcal infections. It causes a wide spectrum of disease pathologies that include CAP, bacterial meningitis, bacteremia, otitis media, sinusitis, septic arthritis, osteomyelitis, peritonitis, and endocarditis owing to its ability to directly extend from the nasopharynx into adjacent anatomic structures or vascular invasion with a hematogenous spread.\(^10\)

Pneumonia is the most common invasive pneumococcal disease, S. pneumoniae being the most frequent cause of bacterial pneumonia due to a known pathogen. It is also suggested to be the most common pathogen in culture-negative disease. However, the actual proportion of CAP caused by S. pneumoniae is difficult to determine given the lack of a gold standard for diagnosis.\(^3\) Up to 60% of individuals in the community have nasopharyngeal carriage of S. pneumoniae.\(^4\)

**Difficulty in Diagnosis and Identification of Streptococcus pneumoniae**

Streptococcus pneumoniae is an important human pathogen having
the potential to cause a broad range of diseases. However, microbiologic confirmation of the pathogen can be difficult. While *S. pneumoniae* has been the most common identifiable pathogen in CAP, there has been a remarkable reduction in the frequency of detection of the pathogen since the 96% diagnostic yield achieved in the pre-antibiotic era, with more recent reports indicating a yield of only 10–20%. Regardless of the potential benefits of the diagnostic tests for *S. pneumoniae*, issues pertaining to both sensitivity and specificity for disease, depending on the clinical setting and type of specimen tested, exist. Interpretation of diagnostic testing for *S. pneumoniae* needs to address the fundamental questions of whether the test in question specifically identifies the pathogen and if the detection adequately implicates it as the causative pathogen of the disease.

Although the most common cause of CAP, it remains undoubtedly underdiagnosed. Isolation from blood is specific but lacks sensitivity, while the isolation from sputum may represent colonization.

*Streptococcus pneumoniae* is identified from the culture by accurate observation of its morphologic appearance and phenotypic characteristics of α-hemolysis of blood agar, optochin susceptibility, catalase negativity, and bile solubility. Although the phenotypic markers are quite reliable, the finding of optochin-resistant pneumococci has decreased the use of this characteristic.

*Streptococcus pneumoniae* is also difficult to obtain in a culture given the tendency of the pathogen to autolyze on reaching the stationary phase of growth, in patients in whom antibiotic treatment has been initiated before collection of specimen, and limitations such as difficulty in adequate collection of specimen and low prevalence of detectable bacteremia in CAP. The first commercialized assays were the tests based on the capsular polysaccharide antigens of *S. pneumoniae*. But these have poor sensitivity and specificity as compared to standard Gram stain and culture.

While empiric therapy for CAP is directed at *S. pneumoniae*, there may be an involvement of other pathogens, such as atypical bacterial pathogens or respiratory viruses. An accurate and reliable finding of *S. pneumoniae* would benefit both pneumococcal and non-pneumococcal disease.

**Summary**

The role of microbiological testing for CAP continues to be debatable. Even with the best available diagnostic methods, a specific etiologic agent is detected in only 50% of CAP cases. The conventional laboratory tests for pathogens causing CAP are so poor that even current clinical practices recommend testing for only the severely affected individuals. It is essential to determine the cause of CAP, whether it is caused by a bacterium, atypical bacterium, or virus, as they all need different treatment approaches. The need for identification of *S. pneumoniae* is essential, as pneumococcal pneumonia is considered to be the main etiology due to cultivable bacteria.

**References**